UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/658,355	09/08/2003	Rene Gantier	17109-013001 / 923	3519

20985 7590 03/12/2007

FISH & RICHARDSON, PC
P.O. BOX 1022
MINNEAPOLIS, MN 55440-1022

EXAMINER	
NEGIN, RUSSELL SCOTT	
ART UNIT	**PAPER NUMBER**
1631	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	03/12/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No. 10/658,355	Applicant(s) GANTIER ET AL.	
	Examiner Russell S. Negin	Art Unit 1631	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 December 2006 and 09 November 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-67 and 79-89* is/are pending in the application.

4a) Of the above claim(s) *12-14,19-27,39-50,56-67 and 82-89* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11,15-18,28-38,51-55 and 79-81* is/are rejected.

7)☒ Claim(s) *1,52 and 55* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7/12/06, 9/26/06, 11/9/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Information Disclosure Statement

The deficiencies of the previous information disclosure statements are addressed by the comments and references filed in the information disclosure statement on 9 November 2006. In addition, the information disclosure statements filed on 12 July 2006 and 26 September 2006 are considered.

Specification

The objections to the specification are withdrawn due to amendments made by applicant to the specification filed on 9 November 2006.

Sequence Compliance

The objections to the specification due to lack of sequence compliance in Figures 6 and 12 of the drawings are withdrawn due to amendments made by applicant to the specification filed on 9 November 2006.

Claim Objections

Claims 1, 52, and 55 are objected to because of the following informalities:

In the third line of step d in claim 1, the word "lead" is in lowercase letters instead of "LEAD."

In the second line of step e in claim 1, the phrase, "from an activity an unmodified target protein," should be "from an activity ***of*** an unmodified target protein."

In the last line of claim 52, the word "Hits" is in lowercase letters instead of "HITs."

In claim 55, the status identifier should have a single closing parenthesis.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The rejections of claims 1-11, 15-18, and 28-38 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention are withdrawn due to amendments made by applicant to the set of claims filed on 6 December 2006.

The rejection of claim 80 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn due to amendments made by applicant to the set of claims filed on 6 December 2006.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-11, 15-18, 28-38, 51-55, and 79-81 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 recites the limitation "the evolved predetermined property or activity" in line 4. There is insufficient antecedent basis for this limitation in the claim. It is assumed that this is a reference to "predetermined property or activity" in line 2 of claim 1. However, this phrase in line 2 is missing the term "evolved." Consequently, the antecedent basis for the phrase "the evolved predetermined property or activity" is ambiguous.

While line 5 of claim 1 states, "wherein each target amino acid is designated as in silico-HIT (is-HIT)," line 4 of step c of claim 1 states, "each replacement is at the same is-HIT locus." It is unclear from these two phrases for independent claims 1, 28 and 51 if an is-HIT is an amino acid or a location of an amino acid in a peptide.

In claim 1, the phrase "single amino acid replacement" occurs several times throughout the claim without a clear relation between its occurrences. For example, the phase occurs on the third lines of both steps b and c. The article preceding "single amino acid," at its first occurrence in step b is "each," while the article preceding the term in the second occurrence is "a." There needs to be more clarification proposed in the claimed subject matter on how these two occurrences of this phrase are related, if related at all.

In claim 1, step a, the last phrase of the claim states, "each target amino acid is designated an in silico-HIT (is-HIT)." However, in step b, the first line states, "identifying one or more replacement amino acids, specific for each is-HIT." In this instance, it is unclear for claims 1, 28, and 51 if each is-HIT is a single amino acid replacement as stated in step a, or possibly a plurality of replacements as indicated in step b.

In claim 1, steps a-c, the term "sets" is used only for nucleic acids, but in step d is used for proteins. It is unclear, when it is used in the independent claims 1, 28 and 51, based on the previous indefiniteness rejections, what is the definition of "set of LEAD proteins." For example, if each LEAD protein in a set contains the same amino acid replacement, it is unclear as to if and how each member in a single set of LEAD proteins differs in composition from other members in the same set. Likewise, the same rejection applies to the definition of "set of candidate super-LEAD" proteins in claims 6 and 33.

It is also unclear in claim 1 where it is stated in step c, "each replacement is at the same is-HIT locus, whereby each candidate LEAD protein is the same," if each candidate LEAD protein has the same amino acid replacement at the same is-HIT locus or if each candidate LEAD protein differs by a single amino acid at the same is-HIT locus.

For the purpose of examination, the claims will be interpreted in light of the specification and arguments, but within the unclear metes and bounds of the claims themselves. For example, an is-HIT will be assumed to be an amino acid locus. A LEAD protein is assumed to be the wild type protein with a single mutation. Each set of LEADs proteins will be assumed to have identical members of LEAD proteins with an identical mutation.

Claim Rejections - 35 USC § 102

The rejections of claims 1, 4-11, 28-35, and 51-52 under 35 U.S.C. 102(b) as being anticipated by Ladner et al. [USPAT 5,096,815] are withdrawn due to arguments proposed by applicants on pages 17-22 of the Remarks of 9 November 2006.

Claim Rejections - 35 USC § 103

The rejections of claims 1, 6, 15-18, 28, 36-38, 52-55, and 81 under 35 U.S.C. 103(a) as being unpatentable over Ladner et al. [USPAT 5,096,815] in view of Alam et al. [Journal of Biotechnology, volume 65, 1998, pages 183-190] are withdrawn due to arguments proposed by applicants on pages 23-25 of the Remarks of 9 November 2006.

The rejections of claims 1-3 under 35 U.S.C. 103(a) as being unpatentable over Ladner et al. [USPAT 5,096,815] in view of Chiang et al. [Annual Reviews of Microbiology, 1999, volume 53, pages 129-154] are withdrawn due to arguments proposed by applicants on pages 21-22 and 25 of the Remarks of 9 November 2006.

The rejections of claims 1 and 79 under 35 U.S.C. 103(a) as being unpatentable over Ladner et al. [USPAT 5,096,815] in view of Jones et al. [CABIOS, volume 8, 1992, pages 275-282] are withdrawn due to arguments proposed by applicants on pages 26-28 of the Remarks of 9 November 2006.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the

invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

35 U.S.C. 103 Rejection #1:

Claims 1, 4-11, 28-35, 51-52, and 80 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ladner et al. [USPAT 5,096,815] in view of Stabach et al. [Biochemistry, 1997, volume 26, pages 57-65].

Claims 1, 4-11, 28-35, 51-52 and 80 state:

1. A method for generating a protein or peptide molecule, having a predetermined property or activity, the method comprising:
(a) identifying, within a target protein or peptide, one or more target amino acids amenable to providing the evolved predetermined property or activity upon amino acid replacement, wherein each target amino acid is designated an in silico-HIT (is-HIT);
(b) identifying, one or more replacement amino acids, specific for each is-HIT, amenable to providing the evolved predetermined property or activity to the target protein upon amino acid replacement, wherein each single amino acid replacement within the target protein or peptide is designated as a candidate LEAD protein;
(c) producing a collection of sets of nucleic acid molecules that encode each of the candidate LEAD proteins, wherein: each encoded candidate LEAD protein contains a single amino acid replacement; each replacement is at the same is-HIT locus, whereby each candidate LEAD protein is the same; each nucleic acid molecule in a set encodes

the same candidate LEAD protein that differs by one amino acid from the target protein or peptide, whereby the members of reach set differ by one amino acid from the members of the other sets; each set is separate from each and all other sets;
(d) individually introducing each set of nucleic acid molecules into a host cells and expressing the encoded candidate LEAD proteins to produce sets of LEAD proteins, wherein: the host cells are in an addressable array such that each lead protein is expressed at a different locus in the array; and
each LEAD protein in a set contains the same amino acid replacement
(e) individually screening each set of encoded candidate LEAD proteins to identify one or more proteins that has an activity that differs from an activity an unmodified target protein, wherein each such identified proteins is designated a LEAD mutant protein.

4. The method of claim 1, wherein the nucleic acid molecules comprise plasmids; and the cells are eukaryotic cells that are transfected with the plasmids or are bacterial cells are transformed with the plasmids.

5. The method of claim 1, wherein the nucleic acid molecules in step (c) are produced by site-specific mutagenesis.

6. The method of claim 1, further comprising: (f) generating a population of sets of nucleic acid molecules encoding a set of candidate super-LEAD proteins, wherein each candidate super-LEAD protein comprises a combination of two or more of the single amino acid mutations derived from two or more LEAD mutant proteins; (g) introducing each set of nucleic acid molecules encoding candidate super-LEADs into cells and expressing the encoded candidate super-LEAD proteins; and (h) individually screening the sets of encoded candidate super-LEAD proteins to identify one or more proteins that has activity that differs from the unmodified target protein and has properties that differ from the original LEADs, wherein each such protein is designated a super-LEAD.

7. The method of claim 6, wherein the nucleic acid molecules in step (f) are produced by a method selected from among Additive Directional Mutagenesis (ADM), multi-overlapped primer extensions, oligonucleotide-mediated mutagenesis, nucleic acid shuffling, recombination, site-specific mutagenesis, and de novo synthesis.

8. The method of claim 1 wherein the is-HITs identified in step (a) correspond to a restricted subset of amino acids along the full length target protein.

9. The method of claim 1, wherein the replacement amino acids identified in step (b) correspond to a restricted subset of the 19 remaining non-native amino acids.

10. The method of claim 1, wherein the nucleic acids of step (c) are produced by systematically replacing each codon that is an is-HIT, with one or more codons encoding a restricted subset of the remaining amino acids, to produce nucleic acid molecules each differing by at least one codon and encoding candidate LEADs.

11. The method of claim 6, wherein the number of LEAD amino acid positions generated on a single nucleic acid molecule is selected from the group consisting of: two, three, four, five, six, seven, eight, nine, ten or more LEAD amino acid positions up to all of the LEAD amino acid positions.

28. A method for generating proteins with a desired property or activity, comprising:
(a) identifying a target protein;
(b) identifying is-HIT target residues associated with the property;
(c) preparing variant nucleic acid molecules encoding variant proteins, wherein each variant nucleic acid encodes a candidate LEAD mutant protein that differs by one replacement amino acid at one locus from the target protein at one is-HIT target residue;
(d) separately introducing the nucleic acid molecules encoding each candidate LEAD protein into hosts for expression thereof, and expressing the nucleic acid molecules encoding each variant protein to produce sets of LEAD proteins, wherein: each candidate LEAD protein in a set contains the same amino acid replacement; each candidate LEAD protein contains a single amino acid replacement; and each replacement is at the same locus;
(e) individually screening each set of variant LEAD candidate proteins to identify any that have an activity or property that differs by a predetermined amount from the activity of the unmodified target protein, thereby identifying proteins that are LEADs.

29. The method of claim 28, wherein either: each of the identified is-HIT target residues in the target protein is replaced with codons encoding a restricted subset of the remaining 19 amino acids; or the total number of is-HIT residues that are replaced with replacement amino acids is less than the total amount of amino acid residues within the full-length of the target protein.

30. The method of claim 28, wherein each of the identified is-HIT residues in the target protein is replaced with codons encoding a restricted subset of the remaining 19 amino acids.

31. The method of claim 28, wherein the total number of is-HIT residues that are replaced with replacement amino acids is less than the total amount of amino acid residues within the full-length of the target protein.

32. The method of claim 28, wherein each of the identified is-HIT residues in the target protein is replaced with codons encoding a restricted subset of the remaining 19 amino acids; and the total number of is-HIT residues that are replaced with replacement amino acids is less than the total amount of amino acid residues within the full-length of the target protein.

33. The method of claim 28, further comprising:

(f) generating a population of sets of nucleic acid molecules encoding a set of candidate super-LEAD proteins, wherein each candidate super-LEAD protein comprises a combination of two or more of the single amino acid mutations derived from two or more LEAD mutant proteins;
(g) introducing each set of nucleic acid molecules encoding candidate super-LEADs into cells and expressing the encoded candidate super-LEAD proteins; and
(h) individually screening the sets of encoded candidate super-LEAD proteins to identify one or more proteins that has activity that differs from the unmodified target protein and has properties that differ from the original LEADs, wherein each such protein is designated a super-LEAD.

34. The method of claim 33, wherein the nucleic acid molecules in step (f) are produced by a method selected from among additive directional mutagenesis (ADM), multi-overlapped primer extensions, oligonucleotide-mediated mutagenesis, nucleic acid shuffling, recombination, site-specific mutagenesis, and de novo synthesis.

35. The method of claim 33, wherein the number of LEAD amino acid positions generated on a single nucleic acid molecule is selected from the group consisting of: two, three, four, five, six, seven, eight, nine, ten or more LEAD amino acid positions up to all of the LEAD amino acid positions.

51. A method for the production of a protein having an evolved property or activity compared to a unmodified target protein, the method comprising:
(a) selecting, on the target protein, one or more target amino acids amenable to providing the evolved property or activity upon amino acid replacement;
(b) replacing each target amino acid with a replacement amino acid amenable to providing the evolved property or activity to form a candidate LEAD protein, wherein only one amino acid replacement occurs on each target protein;
(c) expressing from a nucleic acid molecule each candidate LEAD protein in a cell contained in an addressable array; and
(d) individually assaying each candidate LEAD protein for the presence or absence of the evolved property or activity compared to a unmodified target protein, thereby identifying proteins that are LEADs.

52. The method of claim 51, wherein the selection of the one or more target amino acids in step a) is conducted in silico and the targets amino acids are designated is-Hits.

80. The method of claim 1, wherein the replacement amino acids are pseudo wild-type amino acids, whereby the resulting polypeptide retains activity of the unmodified peptide.

The patent of Ladner et al., entitled, "Generation and selection of novel DNA-binding proteins and polypeptides, states in its abstract

> Novel DNA-binding proteins, especially repressors of gene expression, are obtained by variegation of genes encoding known binding protein and selection for protein binding the desired target DNA sequence.

The computational modeling aspect of the sites for mutagenesis on the proteins are described in the Graphical and Computational Tools section in column 62, line 56 to column 63, line 11 and column 63, lines 25-34, which state,

> The most appropriate method of picking the residues of the protein chain at which the amino acids should be varied is by viewing with interactive computer graphics a model of the initial DBP complexed with operator DNA. This model need not come from an X-ray ray [sic] structure of the complex, but could instead be obtained by docking a 3D structure of the initial DBP to an appropriate model of the DNA operator sequence. A model based on X-ray data from the DNA-protein complex is preferred. A stick figure representation of molecules is preferred. A suitable set of hardware is an Evans & Sutherland PS390 graphics terminal ... and a MicroVAX II supermicro computer... The computer model should preferably have at least 150 megabytes of disk storage, so that the Brookhaven Protein Data Bank can be kept on line. A FORTRAN compiler, or some equally good higher-level language processor is preferred for program development...

Column 63, lines 25-34 of Ladner et al. state,

> In addition, one could use theoretical calculations, such as dynamic simulations of proteins, DNA, or protein-DNA complexes to estimate whether a substitution at a particular residue of a particular amino acid type might produce a protein of approximately the same 3D structure as the parent protein. Such calculations might also indicate whether a particular substitution will greatly affect the flexibility of the protein; calculations of this sort may be useful but are not required.

Empirical methods are described in column 12, lines 50-79, of Ladner et al. which state,

> This application uses the term 'variegated DNA' to refer to a population of molecules that have the same base sequence through most of their length, but that vary at a number of defined loci... When plasmids containing the variegated DNA are used to transform bacteria, each cell makes a version of the original protein. Each colony of bacteria produces a different version from most other colonies. If the variegations of the DNA are concentrated at loci that code on expression for residues known to be on the surface of the protein or in the loops, a population of genes will be generated that code on expression for a population of proteins, many members of which will fold roughly the same 3D structure as the parental protein. Most often we generate mutations that are concentrated within the codons for residues thought to make contact with the DNA.

Empirical methods and assays (which further define a restricted set of "focused" mutations) are further described in columns 77, line 66 to column 78, line 5 of Ladner et al. which states,

> The initial set of 5 residues for Focused Mutagenesis contains residues in or near the N-terminal half of alpha helix 3: Y26, Q27, S28, N31, and K32. Varying these 5 residues through all 20 amino acids produces 3.2×10^6 different protein sequences encoded by 32^5... different DNA sequences. Since all 5 residues are in the same interaction set, the variegation scheme produces the maximum number of different surfaces.

Ladner et al. continue in column 78, lines 17-30 by stating,

> We synthesize DNA inserts having approximate level of variegation, ligate the synthetic DNA into appropriately digested and purified plasmid DNA, transform competent cells, select for uptake of plasmid, introduce the cells for production of potential DBPs [DNA Binding Proteins], and then select for the wanted DNA binding phenotype using the binding marker genes. Surviving colonies are screened genetically and biochemically to verify that a novel DBP is effectively binding to the target DNA sequence. DBP characterization includes, at the genetic level, deleting and back-crossing the dbp gene at the protein level, in vitro binding assays; and, at the DNA level, determination of the complete sequence of the dbp gene.

Consequently, a method for generating a protein or peptide molecule is discussed. Target amino acids are identified by computer, and candidate substitutions are identified. A collection of mutants with desired properties are produced. They are expressed by genes in plasmids in host bacteria, and they are finally screened. This is a process of site-specific mutagenesis at the restricted sited of 5 specific residues. LEAD (single mutation) and super-LEAD (multiple mutation) proteins are produced and screened with in the 3.2×10^6 different protein sequences corresponding to all 20 occurring amino acids. Codons of the genes in the plasmids are altered to result in the mutated proteins. The specific number of amino acids investigated was 5.

Ladner et al. does not show a protein with exclusively a single amino acid mutated at exclusively a single is-HIT locus. Additionally, Ladner et al. does not show each species of mutant peptide being screened individually.

The study of Stabach et al., entitled, "Site-directed mutagenesis of αII spectrin at codon 1175 modulates its μ-Calprin Susceptibility," states in lines 12-13 of the abstract, "Twenty different amino acids were substituted by site-directed mutagenesis for wild-type Val_{1175}, the penultimate (P2) residue flanking the major calpain cleavage site in αII spectrin,"

Stabach et al. states the purpose of this study in the second full paragraph of the first column of page 58 where it is claimed:

> As a first step to exploring the consequences of calpain action specifically on spectrin, human fetal brain all spectrin cDNA was cloned and sequenced, and the determinants of the μ-calpain sensitivity were explored using recombinant αII spectrin peptides in which the Val1175 residue at the P2 position relative to the site of cleavage has been replaced by each of the other 19 amino acids.

Figures 2 and 3 on page 61 of Stabach et al. illustrate the individual screening of each species of mutation with a single amino acid mutated at the 1175 codon as a function of resistance to calpain digestion. Some of the peptides in Figure 3 of Stabach et al. retain the activity of the original peptide.

It would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the combinatorial study of Ladner et al. in view of the single amino acid replacement study of Stabach et al., because while Ladner et al. generates combinatorial mixtures of LEADs, Stabach et al. has the advantage of individually screening each species of singly mutant protein at an identical locus for the purpose of understanding the implications of calpain action on spectrin.

<u>35 U.S.C. 103 Rejection #2</u>:

Claims 1, 6, 15-18, 28, 36-38, 52-55, and 81 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ladner et al. in view of Stabach as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, in further view of Alam et al. [Journal of Biotechnology, volume 65, 1998, pages 183-190].

Claims 6, 15-18, 36-38, 52-55, and 81 state:

6. The method of claim 1, further comprising: (f) generating a population of sets of nucleic acid molecules encoding a set of candidate super-LEAD proteins, wherein each candidate super-LEAD protein comprises a combination of two or more of the single amino acid mutations derived from two or more LEAD mutant proteins; (g) introducing each set of nucleic acid molecules encoding candidate super-LEADs into cells and expressing the encoded candidate super-LEAD proteins; and (h) individually screening the sets of encoded candidate super-LEAD proteins to identify one or more proteins that has activity that differs from the unmodified target protein and has properties that differ from the original LEADs, wherein each such protein is designated a super-LEAD.

15. The method of claim 1, wherein the activity modified is selected from among increased catalytic activity, altered substrate and ligand recognition, increased thermostability, increased stability, increased resistance to proteases, increased resistance to glomerular filtration, increased immunogenicity, increased cationization, increased anionization and pseudo wild-type function.

16. The method of claim 1, wherein each is-HIT target amino acid is susceptible to digestion by one or more proteases.

17. The method of claim 16, wherein the LEADs or super-LEADs possess increased resistance to proteolysis compared to unmodified target protein.

18. The method of claim 1, wherein in a modified protein, each is-HIT target amino acid is resistant to digestion by one or more proteases compared to in unmodified protein.

36. The method of claim 28, wherein each is-HIT target residue is susceptible to digestion by one or more proteases.

37. The method of claim 36, wherein the LEADs or super-LEADs possess increased resistance to proteolysis compared to unmodified target protein.

38. The method of claim 28, wherein each is-HIT target residue is resistant to digestion by one or more proteases.

52. The method of claim 51, wherein the selection of the one or more target amino acids in step a) is conducted in silico and the targets amino acids are designated is-Hits.

53. The method of claim 52, wherein the in silico selection step further comprises selecting an is-HIT target residue that is susceptible to digestion by one or more proteases.

54. The method of claim 53, wherein the LEADs possess increased resistance to proteolysis compared to unmodified target protein.

55. The method of claim 52, wherein the in silico selection step further comprises selecting an is-HIT target residue that is resistant to digestion by one or more proteases.

81. The method of claim 6, wherein the LEADs or super-LEADs possess increased resistance to proteolysis compared to unmodified target protein.

However, Ladner et al. in view of Stabach et al. as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, does not teach increased resistance to proteolysis as a result of mutations.

In the article of Alam et al., entitled, "Expression and purification of a mutant human growth hormone that it resistant to proteolytic cleavage by thrombin, plasmin and human plasma in vitro," Alam et al. take a section of human growth hormone which is not resistant to proteolysis, and conduct mutations to the hormone to make it resistant to proteolysis (see for instant, abstract on page 183 which states, "In this study, oligonucleotide primer-directed mutagenesis was used to produce recombinant mutant hGHs resistant to limited proteolysis by these proteases.")

Alam et al. ends their article in column 2 of page 189 by stating:

> This mutant GH [growth hormone] modified at the proteolytically sensitive sites is expected to have a longer period of bioavailability with characteristic pharmacological importance, implicating a potent clinical application in the future. In that case, however, the possibility that the mutant hGH may raise the antibody during the treatment has to be investigated in detail.

It would have been obvious to someone or ordinary skill in the art at the time of the instant invention to practice Ladner et al. in view of Stabach et al. as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, in further view of Alam et al. to result in the instantly claimed invention because Alam et al. has the advantage of applying the site directed mutagenesis study to proteolysis for the purpose of better understanding of potential antibody production levels.

<u>35 U.S.C. 103 Rejection #3</u>:

Claims 1-3 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ladner et al. in view of Stabach as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, in further view of Chiang et al. [Annual Reviews of Microbiology, 1999, volume 53, pages 129-154].

Claims 2-3 state:

2. The method of claim 1, wherein the array comprises a solid support with separate loci and each set of cells is at a different locus.

3. The method of claim 2, wherein the loci comprise wells; and each well contains one set of cells.

However, Ladner et al. in view of Stabach as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, do not teach use of solid support with wells to analyze each species of protein.

In the article of Chiang et al., "In vivo genetic analysis of bacterial virulence," Chiang et al. state on page 138, last full paragraph, "mutagenized bacterial strains are stored individually in arrays (usually in the wells of microtiter dishes)..."

Chiang et al. end their article in the last three lines of page 149 by stating:

> The value of IVET [in vitro expression technology] and STM [signature tagged mutagenesis] methods in that they allow these types of analysis to be performed simultaneously in a relatively large number of genes during an actual infection.

It would have been obvious to someone or ordinary skill in the art at the time of the instant invention to practice Ladner et al. in view of Stabach as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, in further view of Chiang et al. to result in the instantly claimed invention because Chiang et al. has the advantage of applying the site directed mutagenesis study to the claimed condition of wells on a solid support for a more efficient analysis of gene expression.

<u>35 U.S.C. 103 Rejection #4</u>:

Claims 1 and 79 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ladner et al. in view of Stabach as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, in further view of Jones et al. [CABIOS, volume 8, 1992, pages 275-282].

Claim 79 states:

79. The method of claim 1, wherein the replacement amino acids are selected using Percent Accepted Mutations (PAM) matrices.

However, Ladner et al. in view of Stabach as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, do not teach PAM matrices.

The study of Jones et al., entitled "The rapid generation of mutation data matrices from protein sequences," shows PAM matrices on Table I on page 279 for the purpose of mutation of protein sequences.

Jones et al. explain the purpose of using these specific techniques at the end of the third full paragraph of column 1 of page 276:

> ...it is our hope that the matrices presented here will more clearly express the general nature of the underlying amino acid similarities.

It would have been obvious to someone or ordinary skill in the art at the time of the instant invention to practice Ladner et al. in view of Stabach as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, in further view of Jones et al. to result in the instantly claimed invention because Jones et al. has the advantage of applying the site directed mutagenesis study to the claimed analysis condition of PAM matrices for the purpose of a clearer and more efficient understanding of the amino acid residues comprising the protein of interest.

Double Patenting

The provisional rejections of claims 1, 4-11, 15, and 79-81 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 250, 251, 256-264, and 268-269 of copending Application No. 10/658,834 are withdrawn based on cancellation of the corresponding claims in the copending U.S. application.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

The rejections of claims 1, 4-11, 15, and 79-81 under 35 U.S.C. 102(f) because the applicant did not invent the claimed subject matter are withdrawn due to the content of the declaration filed on 9 November 2006.

Response to Arguments

Applicant's arguments filed 9 November 2006 have been fully considered but they are partially persuasive. The combination of the arguments and the amendments to the claims filed on 6 December 2006 result in the new prior art rejections in this Office action.

With regards to the previous anticipatory prior art rejection, applicants' arguments listed on pages 21-22 of the Remarks of 9 November 2006 are based on two aspects:

First, applicants claim that Ladner et al. does not teach modification at exclusively a single residue.

Second, applicants claim that Ladner et al. does not teach that each polypeptide is expressed and screened separately.

These arguments are addressed by combining the teaching of Ladner et al. in view of the teachings of Stabach et al.

The arguments of the obviousness prior art rejections are based on deficiencies in the withdrawn anticipatory prior art rejection and are also addressed by adding the reference of Stabach et al. to the respective obviousness prior art rejections.

Conclusion

No claim is allowed.

Papers related to this application may be submitted to Technical Center 1600 by facsimile transmission. Papers should be faxed to Technical Center 1600 via the central PTO Fax Center. The faxing of such pages must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993)(See 37 CFR § 1.6(d)). The Central PTO Fax Center Number is (571) 273-8300.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Russell Negin, Ph.D., whose telephone number is (571) 272-1083. The examiner can normally be reached on Monday-Friday from 7am to 4pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's Supervisor, Irem Yucel, Supervisory Patent Examiner, can be reached at (571) 272-0781.

Information regarding the status of the application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information on the PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

RSN
5 March 2007

5 march 2007

5 March 2007

JOHN S. BRUSCA, PH.D
PRIMARY EXAMINER